

## FIG. 1A

pET22b(+) forward primer:

5'-CGGGATCCT TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:3)

pET22b(+) reverse primer:

5'-CCCAAGCTT TGT TCT TCT CAT ACA GAC-3' (SEQ ID NO:4)

pPICZαA forward primer:

5'-TTCGGAATTC TCT GTT GAT CAC GGC TTC-3' (SEQ ID NO:15)

pPICZαA reverse primer:

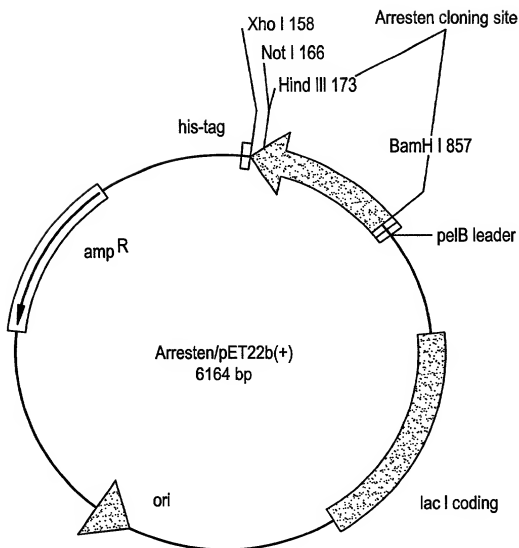
5'-TGCTCTAGAGG TGT TCT TCT CAT ACA GAC TTG GCA-3' (SEQ ID NO:16)

|  |     |     |     |     |     |     |     |     |     |
|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| 5  | 10  | 15  | 20  | 25  | 30  | 35  | 40  | 45  |     |
| <u>tct gtt gat cac ggc ttc</u>                                     | ctt | gtg | acc | agg | cat | agt | caa | aca | ata |
| 50   | 55  | 60  | 65  | 70  | 75  | 80  | 85  | 90  |     |
| gat gac cca cag tgt cct tct ggg acc aaa att ctt tac cac ggg        |     |     |     |     |     |     |     |     |     |
| 95   | 100 | 105 | 110 | 115 | 120 | 125 | 130 | 135 |     |
| tac tct ttg ctg tac gtg caa ggc aat gaa cgg gcc cat gga cag        |     |     |     |     |     |     |     |     |     |
| 140  | 145 | 150 | 155 | 160 | 165 | 170 | 175 | 180 |     |
| gac ttg ggc acg gcc ggc agc tgc ctg cgc aag ttc agc aca atg        |     |     |     |     |     |     |     |     |     |
| 185  | 190 | 195 | 200 | 205 | 210 | 215 | 220 | 225 |     |
| ccc ttc ctg ttc tgc aat att aac aac gtg tgc aac ttt gca tca        |     |     |     |     |     |     |     |     |     |
| 230  | 235 | 240 | 245 | 250 | 255 | 260 | 265 | 270 |     |
| cga aat gac tac tgc tac tgg ctg tcc acc cct gag ccc atg ccc        |     |     |     |     |     |     |     |     |     |
| 275  | 280 | 285 | 290 | 295 | 300 | 305 | 310 | 315 |     |
| atg tca atg gca ccc atc acg ggg gaa aac ata aga cca ttt att        |     |     |     |     |     |     |     |     |     |
| 320  | 325 | 330 | 335 | 340 | 345 | 350 | 355 | 360 |     |
| agt agg tgt gct gtg tgt gag gcg cct gcc atg gtg atg gcc gtg        |     |     |     |     |     |     |     |     |     |
| 365  | 370 | 375 | 380 | 385 | 390 | 395 | 400 | 405 |     |
| cac agc cag acc att cag atc cca ccg tgc ccc agc ggg tgg tcc        |     |     |     |     |     |     |     |     |     |
| 410  | 415 | 420 | 425 | 430 | 435 | 440 | 445 | 450 |     |
| tcg ctg tgg atc ggc tac tct ttt gtg atg cac acc agc gct ggt        |     |     |     |     |     |     |     |     |     |
| 455  | 460 | 465 | 470 | 475 | 480 | 485 | 490 | 495 |     |
| gca gaa ggc tct ggc caa gcc ctg gcg tcc ccc ggc tcc tgc ctg        |     |     |     |     |     |     |     |     |     |
| 500  | 505 | 510 | 515 | 520 | 525 | 530 | 535 | 540 |     |
| gag gag ttt aga agt gcg cca ttc atc gag tgt cac ggc cgt ggg        |     |     |     |     |     |     |     |     |     |
| 545  | 550 | 555 | 560 | 565 | 570 | 575 | 580 | 585 |     |
| acc tgc aat tac tac gca aac gct tac agc ttt tgg ctg gcc acc        |     |     |     |     |     |     |     |     |     |
| 590  | 595 | 600 | 605 | 610 | 615 | 620 | 625 | 630 |     |
| ata gag agg agc gag atg ttc aag aag cct acg ccg tcc acc ttg        |     |     |     |     |     |     |     |     |     |
| 635  | 640 | 645 | 650 | 655 | 660 | 665 | 670 | 675 |     |
| aag gca ggg gag ctg cgc acg cac gtc agc cgc <u>tgc caa gtc tgt</u> |     |     |     |     |     |     |     |     |     |
| 680  | 685 | 690 |     |     |     |     |     |     |     |
| <u>atg aga aga aca</u>   | taa |     |     |     |     |     |     |     |     |

(SEQ ID NO:1)

FIG. 1B

FIG. 2



Forward primer: 5'-cgggatccttctgttgatcacggcttc-3'

Reverse primer: 5'-ccaagcttggttcttcatacagac-3'

FIG. 3A

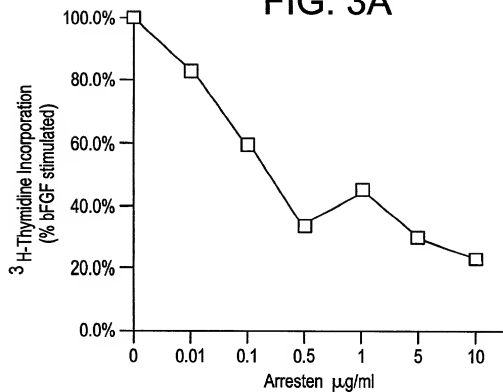


FIG. 3B

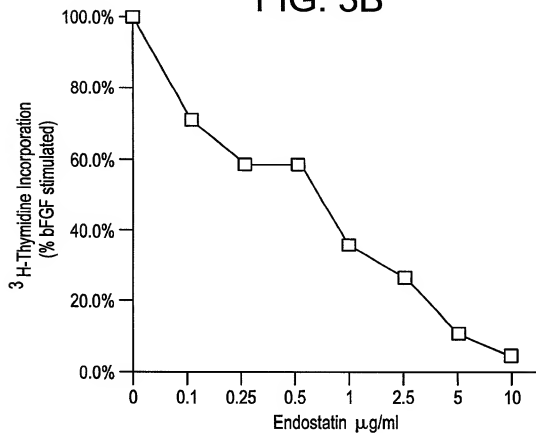


FIG. 4A

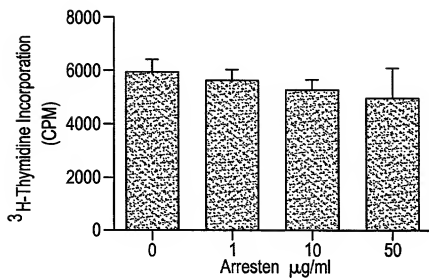


FIG. 4B

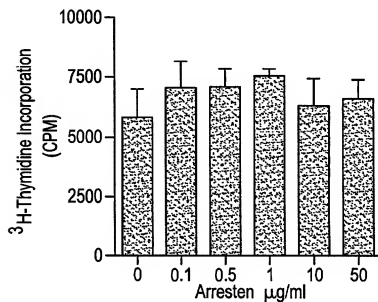


FIG. 4C

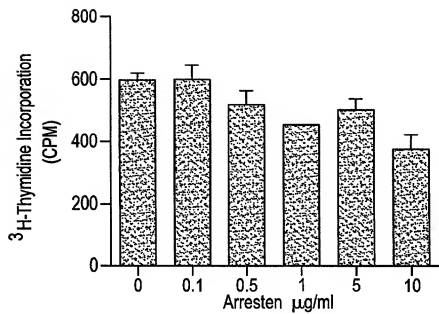


FIG. 4D

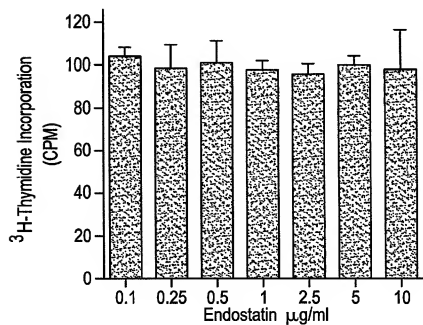


FIG. 5A

Control

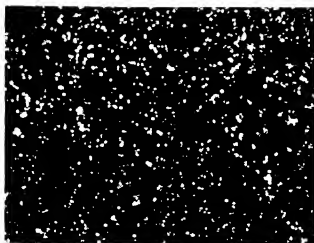


FIG. 5B

Arresten 2  $\mu$ g/ml



FIG. 5C

Endostatin 20  $\mu$ g/ml



FIG. 6

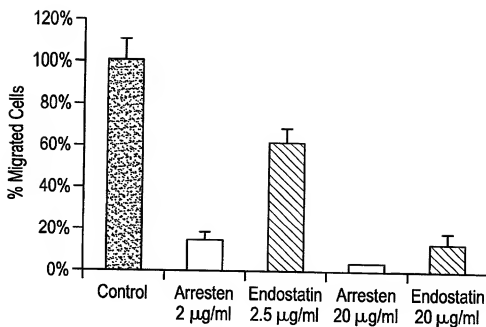


FIG. 7

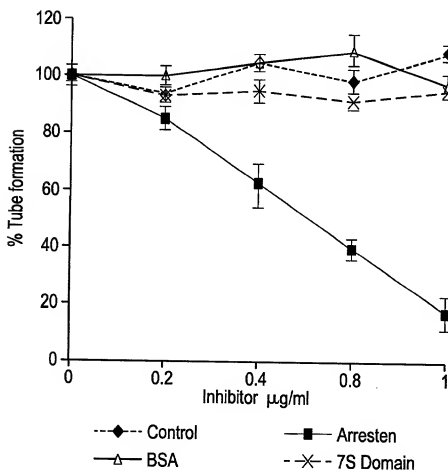


FIG. 8A

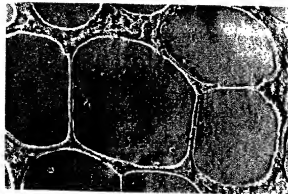


FIG. 8B



FIG. 9A

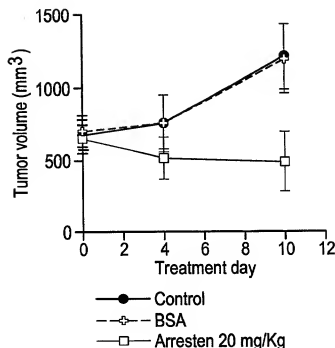


FIG. 9B

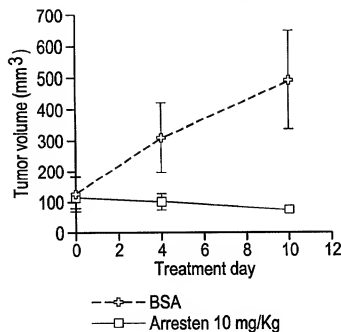


FIG. 9C

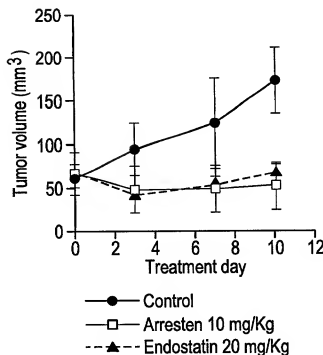
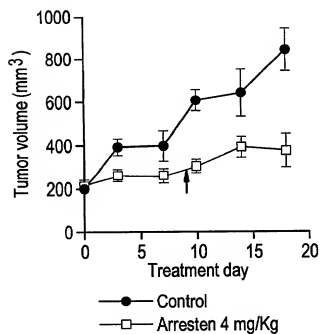


FIG. 9D



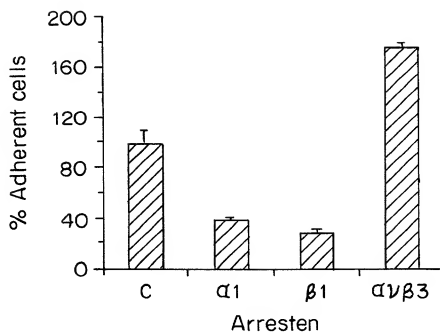


FIG. IOA

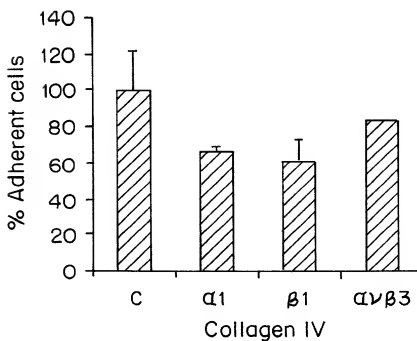


FIG. IOB

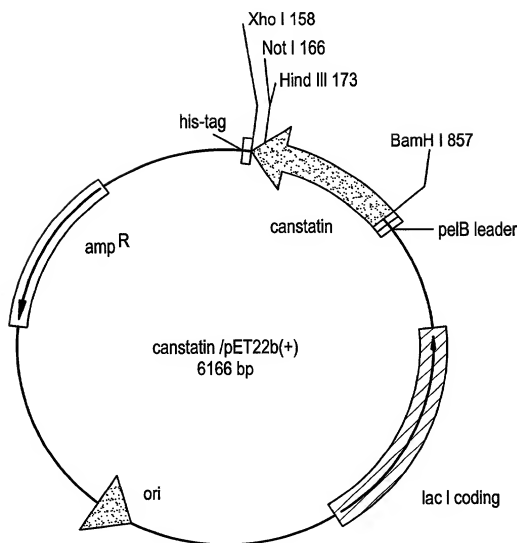


## FIG. 11B

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|     | 5   | 10  | 15  | 20  | 25  | 30  | 35  | 40  | 45  |     |     |     |     |     |
| VSI | GYL | LVK | HSQ | TDQ | EPM | CPV | GMN | KLW | SGY | SLL | YFE | GQE | KAH | NQD |
|     | 50  | 55  | 60  | 65  | 70  | 75  | 80  | 85  | 90  |     |     |     |     |     |
| LGL | AGS | CLA | RFS | TMP | FLY | CNP | GDV | CYY | ASR | NDK | SYW | LST | TAP | LPM |
|     | 95  | 100 | 105 | 110 | 115 | 120 | 125 | 130 | 135 |     |     |     |     |     |
| MPV | AED | EIK | PYI | SRC | SVC | EAP | AIA | IAV | HSQ | DVS | IPH | CPA | GWR | SLW |
|     | 140 | 145 | 150 | 155 | 160 | 165 | 170 | 175 | 180 |     |     |     |     |     |
| IGY | SFL | MHT | AAG | DEG | GGQ | SLV | SPG | SCL | EDF | RAT | PFI | ECN | GGR | GTC |
|     | 185 | 190 | 195 | 200 | 205 | 210 | 215 | 220 | 225 |     |     |     |     |     |
| HYY | ANK | YSF | WLT | TIP | EQS | FQG | SPS | ADT | LKA | GLI | RTH | ISR | CQV | CMK |

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NL (SEQ ID NO:6)

FIG. 12



Forward primer: 5'-cgggatcctgtcagcatcggtacctc-3'

Reverse primer: 5'-cccaagcttcaggcttctcatgcacac-3'

FIG. 13A

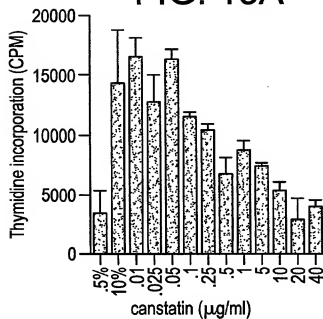


FIG. 13B

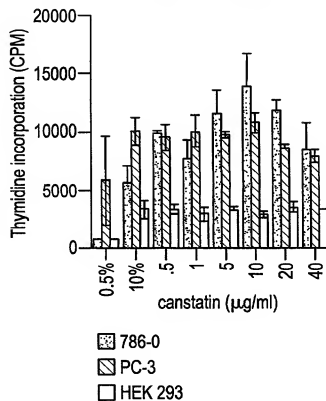


FIG. 13C

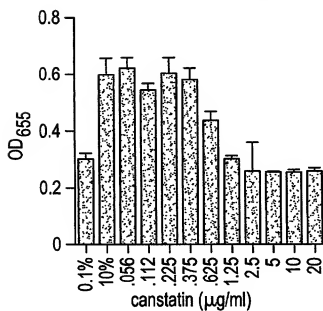


FIG. 13D

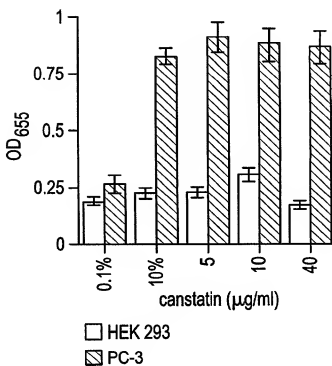


FIG. 14

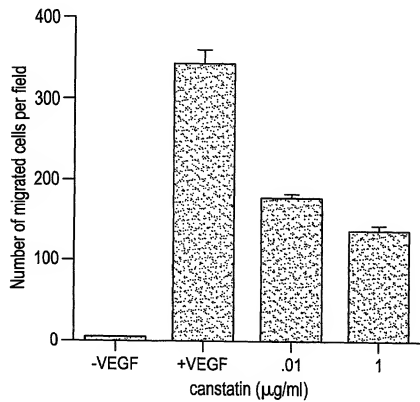
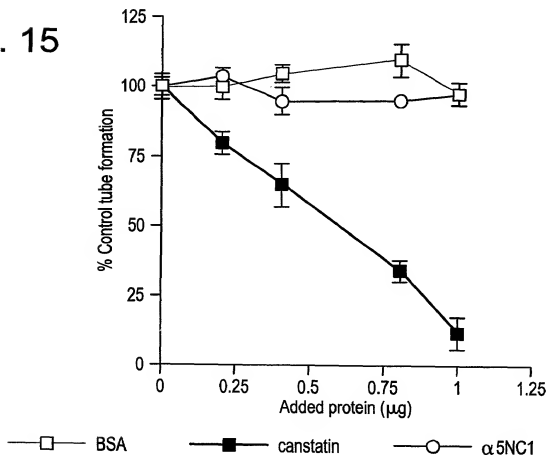


FIG. 15



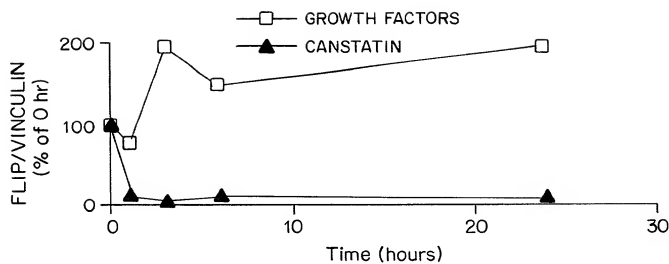


FIG. 16

FIG. 17A

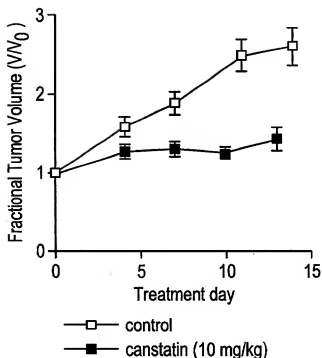


FIG. 17B

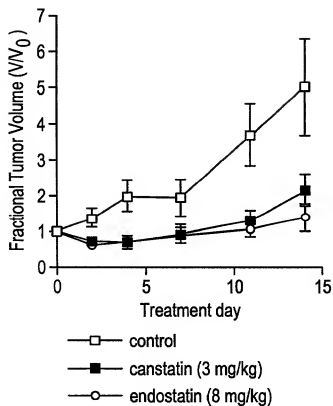


FIG. 17C

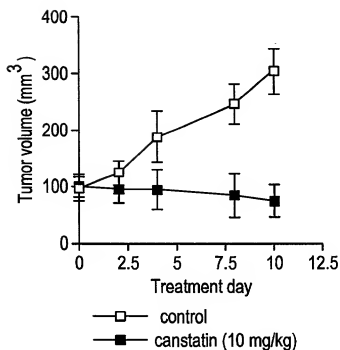
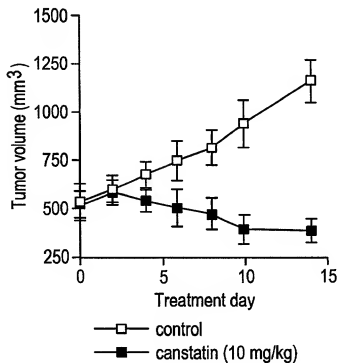


FIG. 17D



## FIG. 18A

pET22b(+) forward primer:

5'-CGGGAT CCA GGT TTG AAA GGA AAA CGT-3' (SEQ ID NO:11)

pET22b(+) reverse primer:

5'-CCCAAGCTT TCA GTG TCT TTT CTT CAT-3' (SEQ ID NO:12)

|                                    |             |             |         |         |         |         |     |     |
|------------------------------------|-------------|-------------|---------|---------|---------|---------|-----|-----|
| 5                                  | 10          | 15          | 20      | 25      | 30      | 35      | 40  | 45  |
| <u>cga ggt tta aaa gga aaa cgt</u> | gga gac agt | gga tca cct | gca acc |         |         |         |     |     |
| 50                                 | 55          | 60          | 65      | 70      | 75      | 80      | 85  | 90  |
| tgg aca acg                        | aga ggc     | ttt gtc     | tto acc | cga cac | agt caa | acc aca |     |     |
| 95                                 | 100         | 105         | 110     | 115     | 120     | 125     | 130 | 135 |
| gca att cct                        | tca tgt     | cca gag     | ggg aca | gtg cca | ctc tac | agt ggg |     |     |
| 140                                | 145         | 150         | 155     | 160     | 165     | 170     | 175 | 180 |
| ttt tct ttt                        | ctt ttt     | gta caa     | gga aat | caa cga | gcc cac | gga caa |     |     |
| 185                                | 190         | 195         | 200     | 205     | 210     | 215     | 220 | 225 |
| gac ctt gga                        | act ctt     | ggc agc     | tgc ctg | cag cga | ttt acc | aca atg |     |     |
| 230                                | 235         | 240         | 245     | 250     | 255     | 260     | 265 | 270 |
| cca ttc tta                        | ttc tgc     | aat gtc     | aat gat | gta tgt | aat ttt | gca tct |     |     |
| 275                                | 280         | 285         | 290     | 295     | 300     | 305     | 310 | 315 |
| cga aat gat                        | tat tca     | tac tgg     | ctg tca | aca cca | gct ctg | atg cca |     |     |
| 320                                | 325         | 330         | 335     | 340     | 345     | 350     | 355 | 360 |
| atg aac atg                        | gct ccc     | att act     | ggc aga | gcc ctt | gag cct | tat ata |     |     |
| 365                                | 370         | 375         | 380     | 385     | 390     | 395     | 400 | 405 |
| agc aga tgc                        | act gtt     | tgt gaa     | ggt cct | gcg atc | gcc ata | gcc gtt |     |     |
| 410                                | 415         | 420         | 425     | 430     | 435     | 440     | 445 | 450 |
| cac agc caa                        | acc act     | gac att     | cct cca | tgt cct | cac ggc | tgg att |     |     |
| 455                                | 460         | 465         | 470     | 475     | 480     | 485     | 490 | 495 |
| tct ctc tgg                        | aaa gga     | ttt tca     | tto atc | atg ttc | aca agt | gca ggt |     |     |
| 500                                | 505         | 510         | 515     | 520     | 525     | 530     | 535 | 540 |
| tct gag ggc                        | acc ggg     | caa gca     | ctg gcc | tcc cct | ggc tcc | tgc ctg |     |     |
| 545                                | 550         | 555         | 560     | 565     | 570     | 575     | 580 | 585 |
| gaa gaa ttc                        | cga gcc     | agc cca     | ttt cta | gaa tgt | cat gga | aga gga |     |     |
| 590                                | 595         | 600         | 605     | 610     | 615     | 620     | 625 | 630 |
| acg tgc aac                        | tac tat     | tca aat     | tcc tac | agt ttc | tgg ctg | gct tca |     |     |
| 635                                | 640         | 645         | 650     | 655     | 660     | 665     | 670 | 675 |
| tta aac cca                        | gaa aga     | atg ttc     | aga aag | cct att | cca tca | act gtg |     |     |
| 680                                | 685         | 690         | 695     | 700     | 705     | 710     | 715 | 720 |
| aaa gct ggg                        | gaa tta     | gaa aaa     | ata ata | agt cgc | tgt cag | gtg tgc |     |     |
| 725                                | 730         | 735         |         |         |         |         |     |     |
| <u>atg aag aaa aga cac tga</u>     |             |             |         |         |         |         |     |     |

(SEQ ID NO:9)

pET22b- $\alpha$ 3(IV) NC1 = nucleotides 4 through 735

Tumstatin 333 = nucleotides 4 through 375

Tumstatin 334 - nucleotide 376 through 735

|     |     |     |     |     |     |     |                |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|----------------|-----|-----|-----|-----|-----|-----|-----|
| *   | 5   | 10  | 15  | 20  | 25  | 30  | 35             | 40  | 45  |     |     |     |     |     |
| PGL | KGK | RGD | SGS | PAT | WTT | RGF | VFT            | RHS | QTT | AIP | SCP | EGT | VPL | YSG |
|     | 50  | 55  | 60  | 65  | 70  | 75  | 80             | 85  | 90  |     |     |     |     |     |
| FSF | LFV | QGN | QRA | HGQ | DLG | TLG | SCL            | QRF | TTM | PFL | FCN | VND | VCN | PAS |
|     |     |     |     |     |     |     |                |     |     |     | *+  |     |     |     |
|     | 95  | 100 | 105 | 110 | 115 | 120 | 125            | 130 | 135 |     |     |     |     |     |
| RND | YSY | WLS | TPA | LMP | MNM | API | TGR            | ALE | PYI | SRC | TVC | EGP | AIA | IAV |
|     |     |     |     |     |     |     |                |     |     |     |     |     |     |     |
|     | 140 | 145 | 150 | 155 | 160 | 165 | 170            | 175 | 180 |     |     |     |     |     |
| HSQ | TTD | IPP | CPH | GWI | SLW | KGF | SFI            | MFT | SAG | SEG | TGQ | ALA | SPG | SCL |
|     |     |     |     |     |     |     |                |     |     |     |     |     |     |     |
|     | 185 | 190 | 195 | 200 | 205 | 210 | 215            | 220 | 225 |     |     |     |     |     |
| EEF | RAS | PFL | ECH | GRG | TCN | YYS | NSY            | SFW | LAS | LNP | ERM | FRK | PIP | STV |
|     |     |     |     |     |     |     |                |     |     |     |     |     |     |     |
|     | 230 | 235 | 240 | 245 |     |     |                |     |     |     |     |     |     |     |
| KAG | ELE | KII | SRC | QVC | MKK | RH  | (SEQ ID NO:10) |     |     |     |     |     |     |     |

pET22b  $\alpha 3(\text{IV})$  NC1 = residues 2 through 245

Tumstatin 333 = residues 2 through 125

Tumstatin 334 = residues 126 through 245

FIG. 19

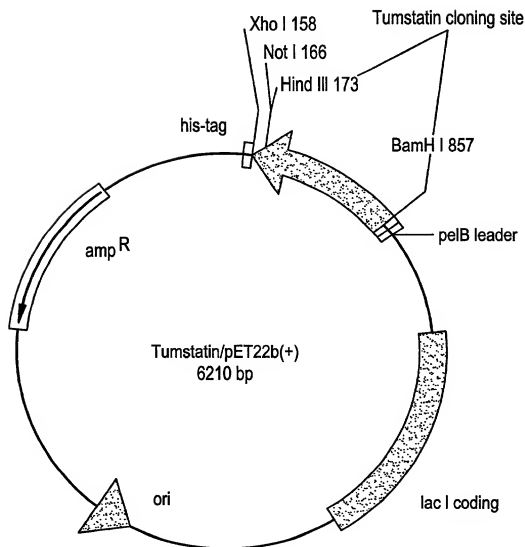


FIG. 20

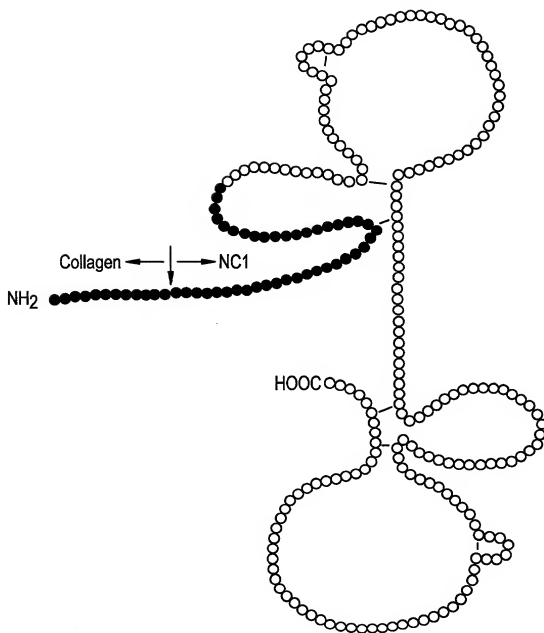


FIG. 21C

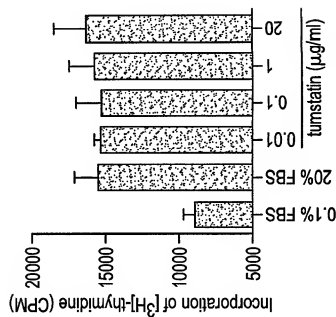


FIG. 21B

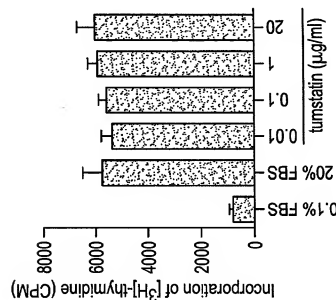
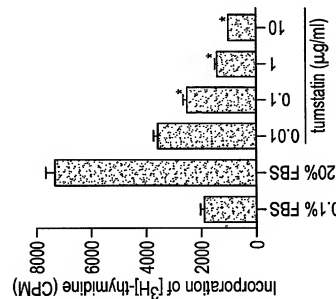


FIG. 21A



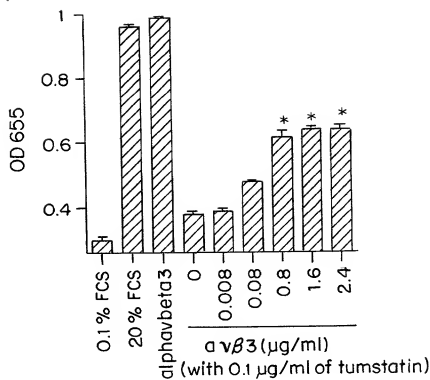


FIG. 22

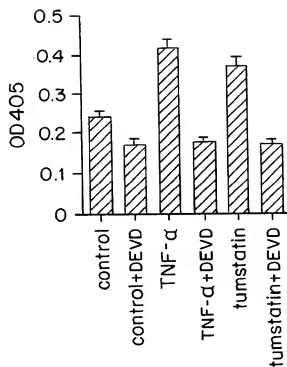


FIG. 23A

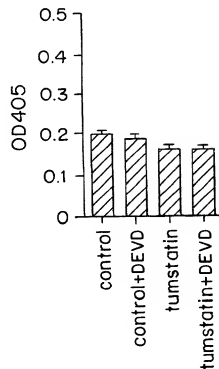


FIG. 23B

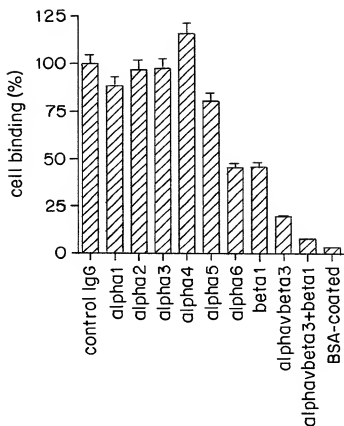


FIG. 24A

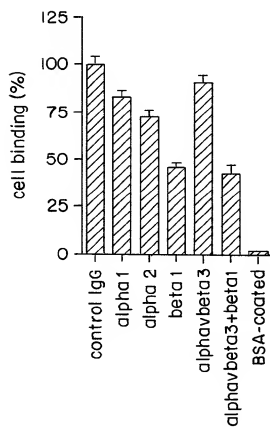


FIG. 24B

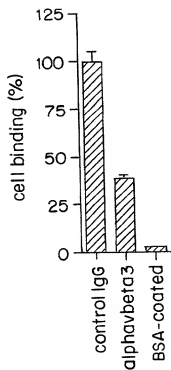


FIG. 24C

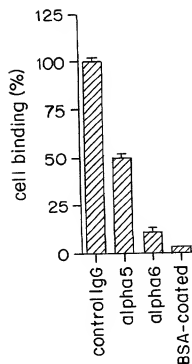


FIG. 24D

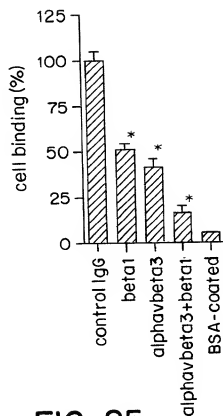


FIG. 25

FIG. 26

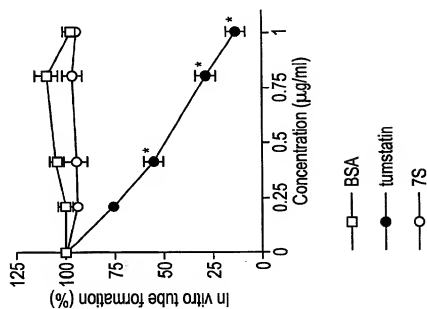


FIG. 27A

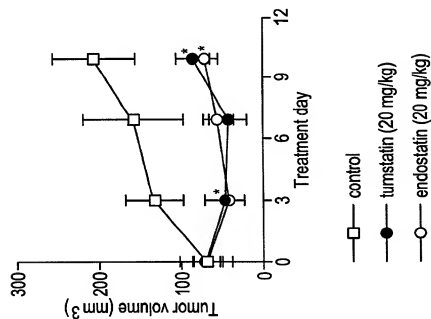


FIG. 27B

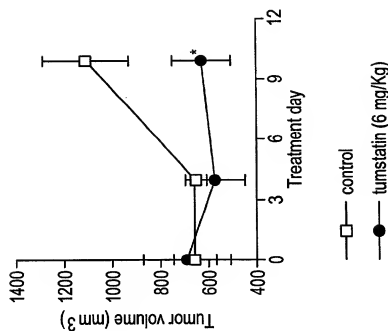
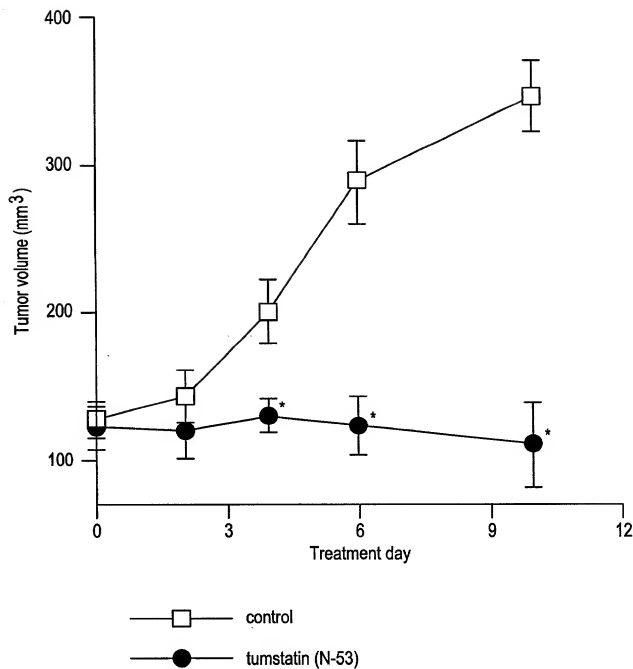


FIG. 28



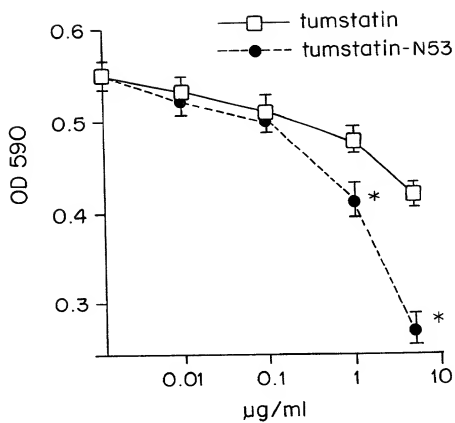


FIG. 29

FIG. 30

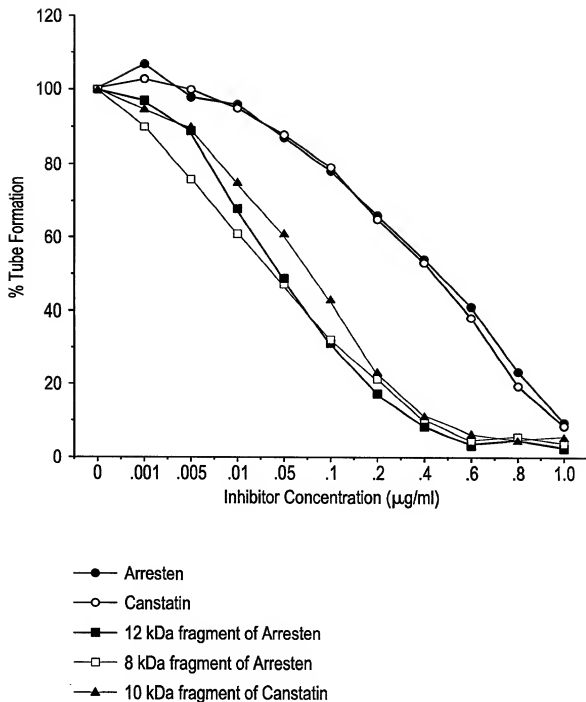
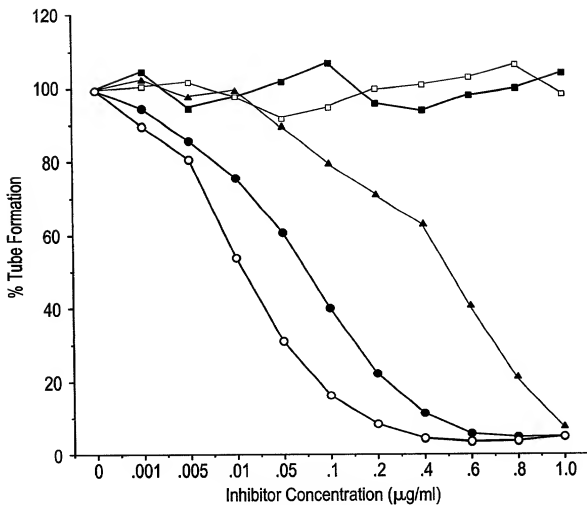
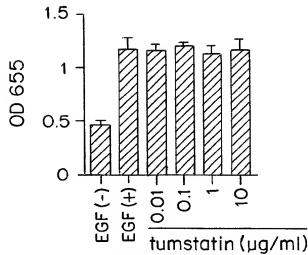


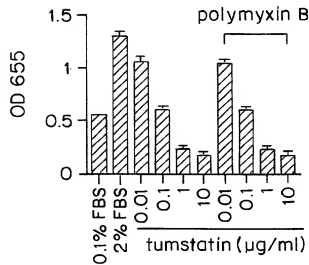
FIG. 31



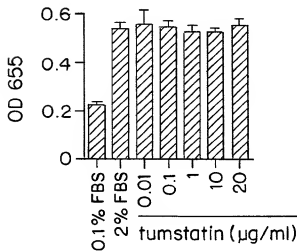
- Tumstatin Fragment 333
- Tumstatin Fragment 334
- BSA
- α6
- ▲ Tumstatin



**FIG. 32A**



**FIG. 32B**



**FIG. 32C**

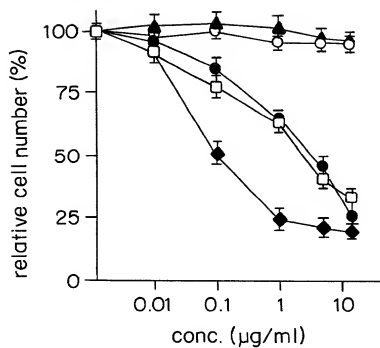


FIG. 33A

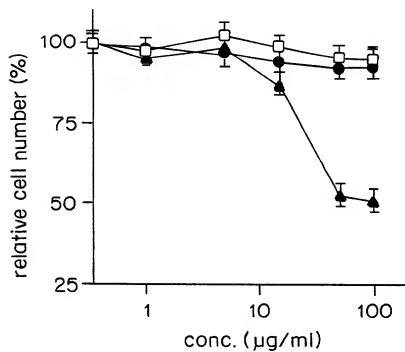


FIG. 33B

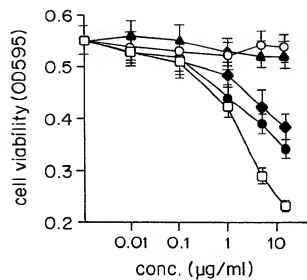


FIG. 34A

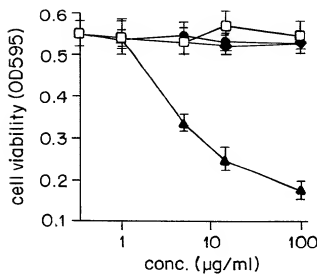


FIG. 34B

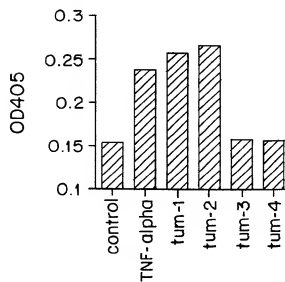


FIG. 35

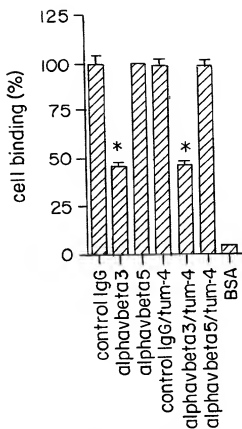


FIG. 36A

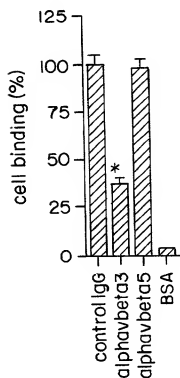


FIG. 36C

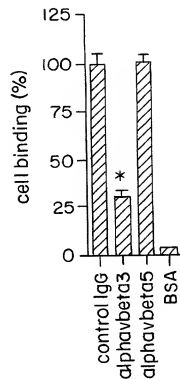


FIG. 36B

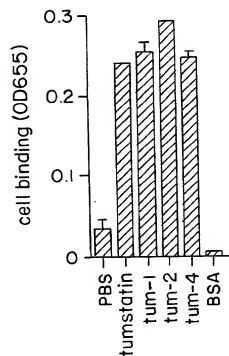


FIG. 37

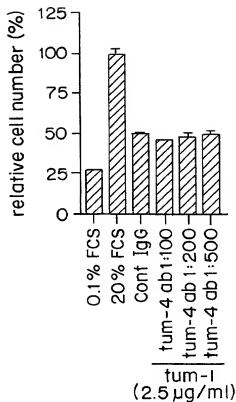


FIG. 38A

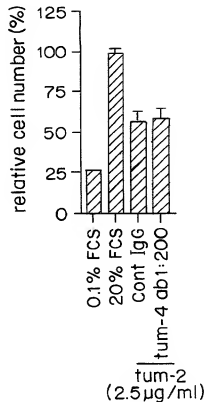


FIG. 38B

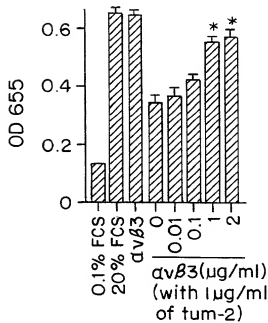


FIG. 38C

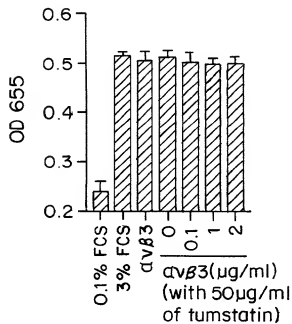


FIG. 38D

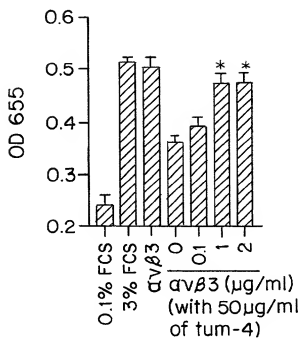


FIG. 38E

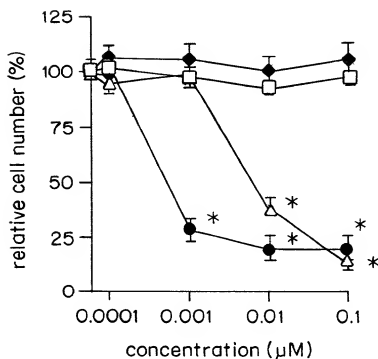


FIG. 39

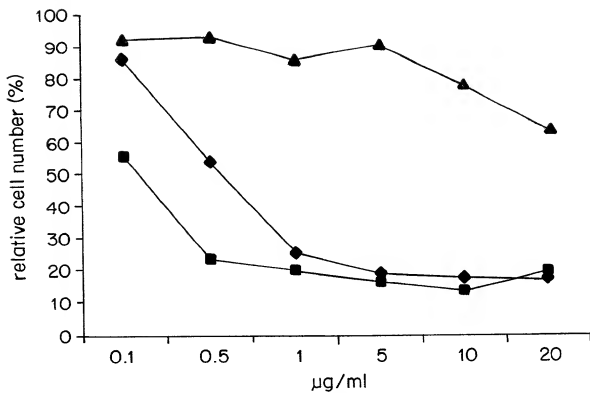


FIG. 40

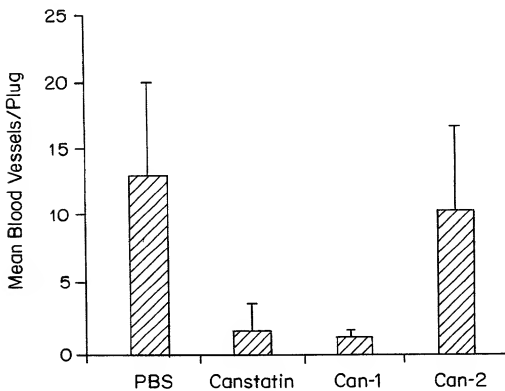


FIG. 41

## FIG. 42

T1 GP-A  
PGLKGK**RGD**SGSPATWTTRGFVFTRHSQT**TAIPSC**PEGTVP**LY**

SGFSFLFVQGNQRAHGQDLGTLGSC**LQRFT**TM**PFLFC**NVNDVC  
T2 T3 T4

T3 T5 T6  
T4  
NFASRNDYSYWLSTPALMPMNMAPITGRALEPYISRCTVCEGP

T6 GP-B  
AIAIAVHSQTT**DIPPC**PHGWISLWKGFSFIMFTSAGSEGTGQA  
LASPGSCLEEFRA**SPFLE**CHGRGTCNYYSNSYSFWLASLNPER  
MFRKPI**PSTV**KAGELEKIISRCQVCMKKRH

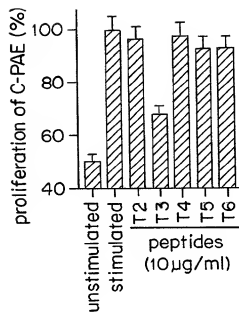


FIG. 43A

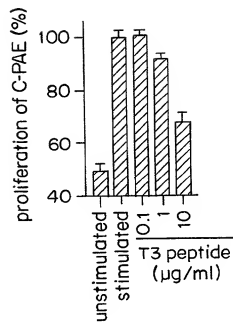


FIG. 43B

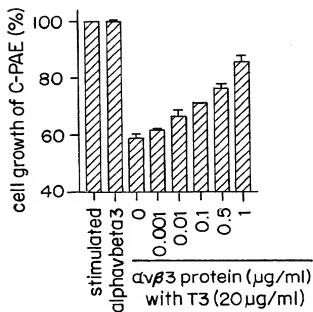


FIG. 43C

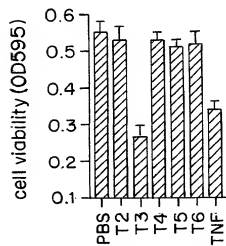


FIG. 43D

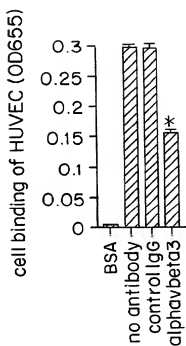


FIG. 44A

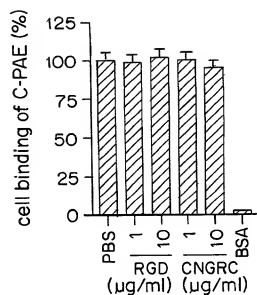


FIG. 44B

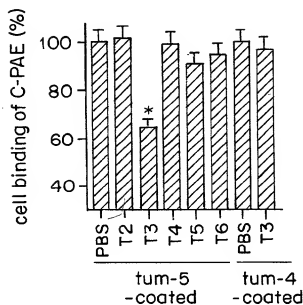


FIG. 44C

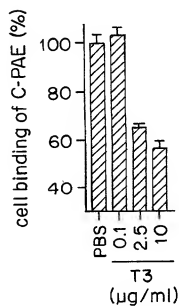


FIG. 44D

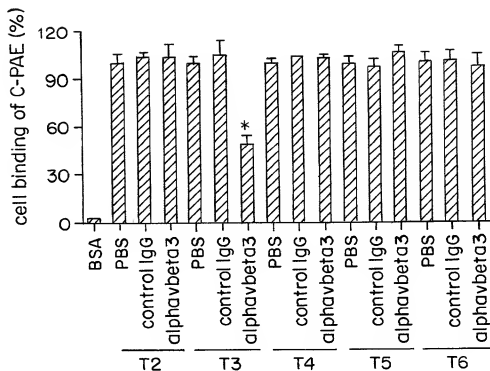


FIG. 44E

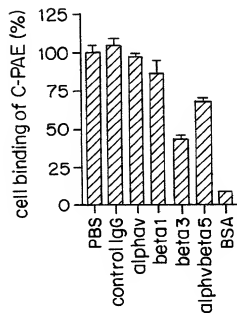


FIG. 44F

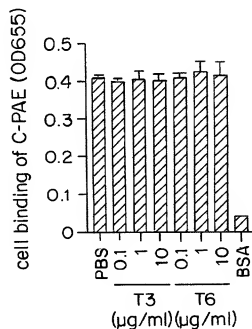


FIG. 44G

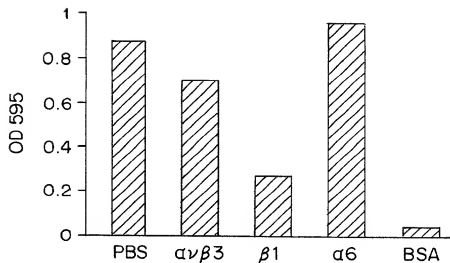


FIG. 45

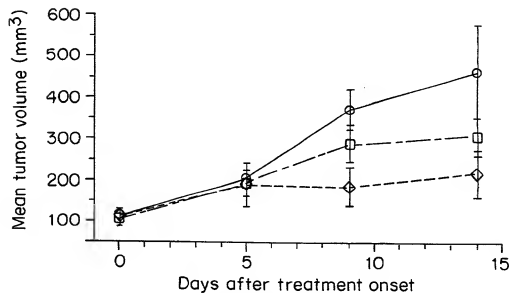


FIG. 46

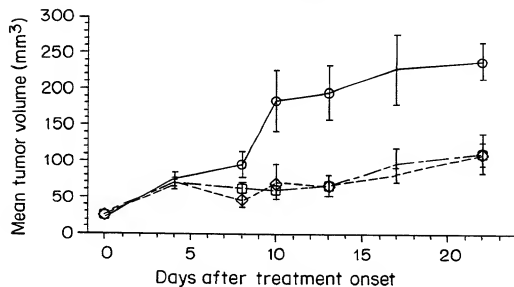


FIG. 47

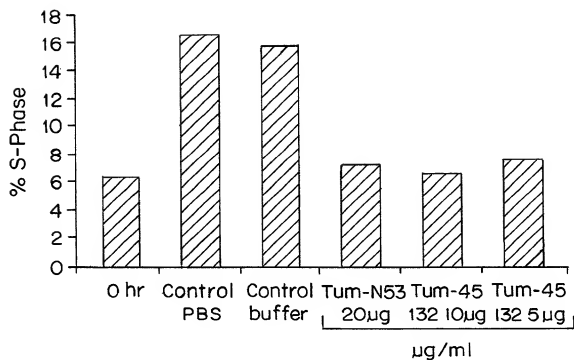


FIG. 48

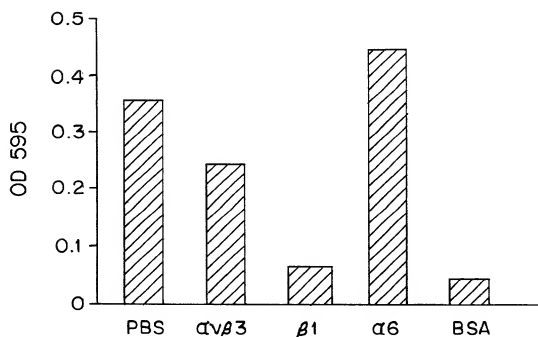


FIG. 49

Fig. 50

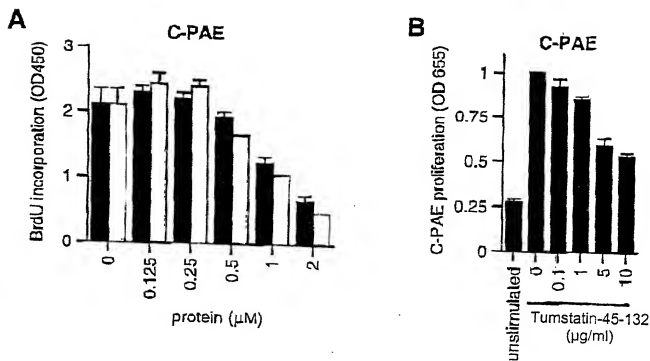


Fig. 51

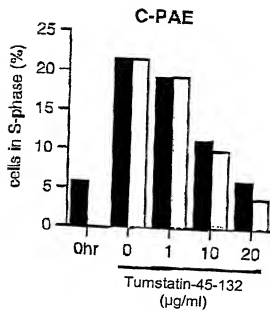


Fig. 52

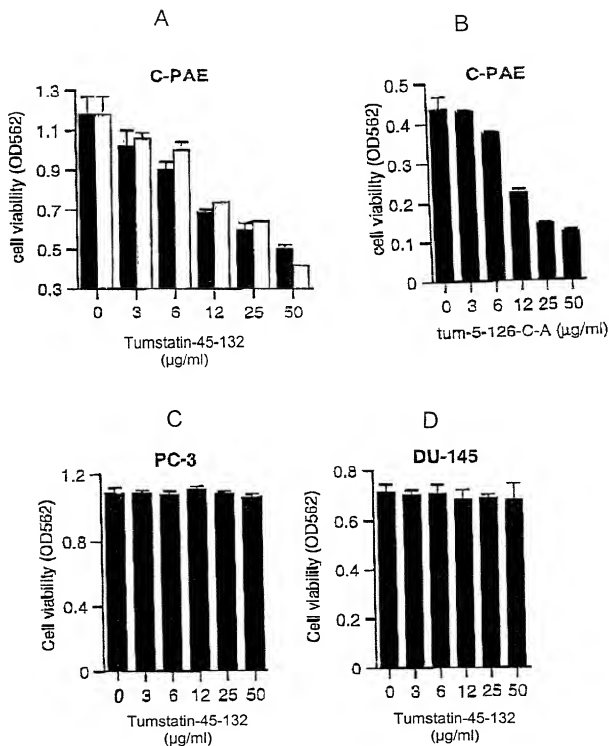


Fig. 53

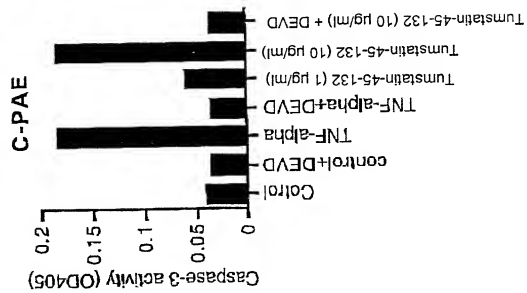
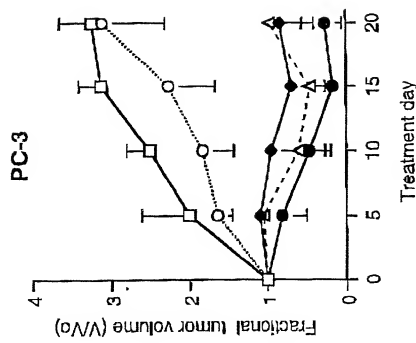


Fig. 54



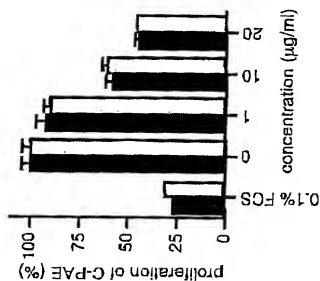


Fig. 56

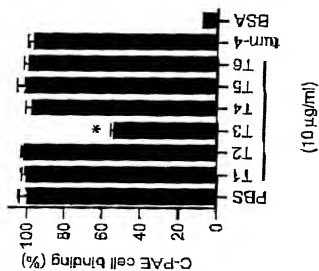


Fig. 55A



Fig. 55B

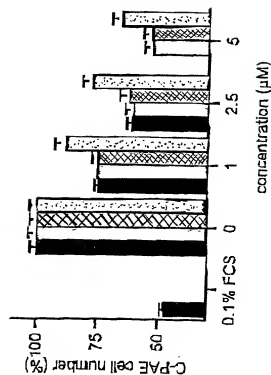


Fig. 57

Fig. 58A

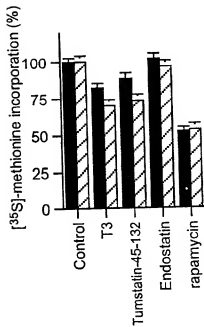


Fig. 58B

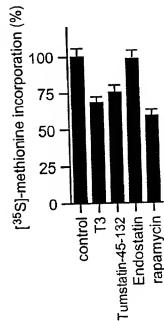


Fig. 58C

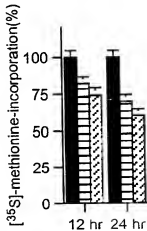


Fig. 58D

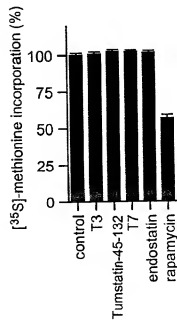


Fig. 58E

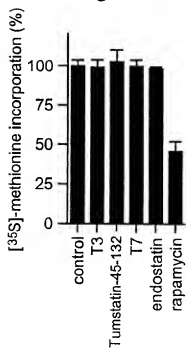


Fig. 58F

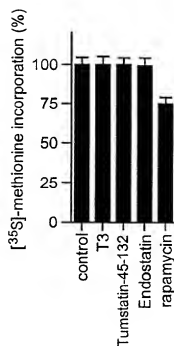


Fig. 58G

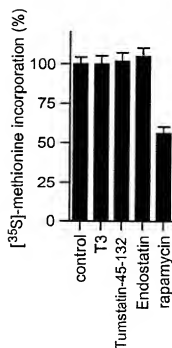


Fig. 58H

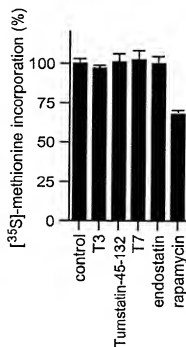


Fig. 59A

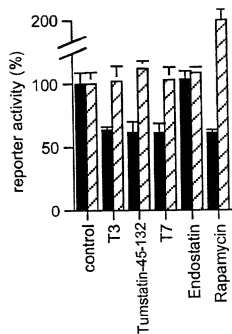


Fig. 59B

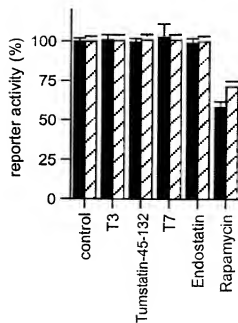


Fig. 60A

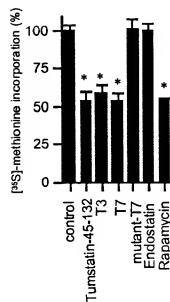


Fig. 60B

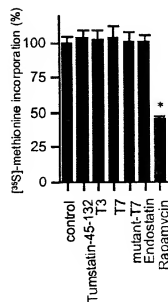


Fig. 60C

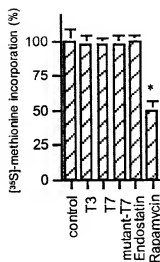


Fig. 60D

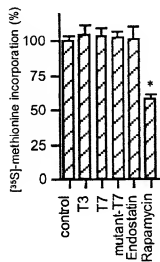


Fig. 60E

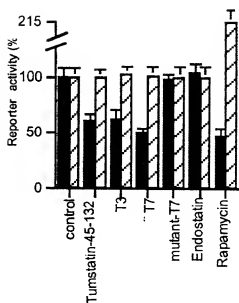


Fig. 60F

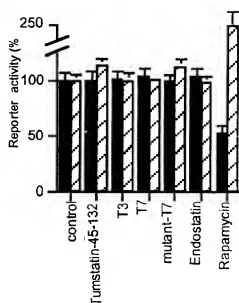


Fig. 60G

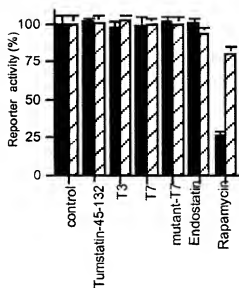


Fig. 60H

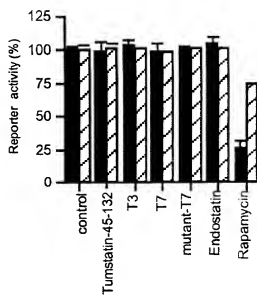


Fig. 61A

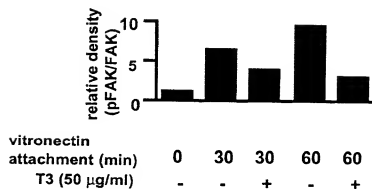


Fig. 61B

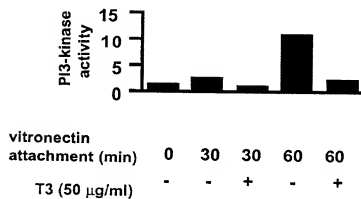


Fig. 61C

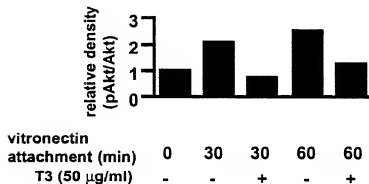


Fig. 61D

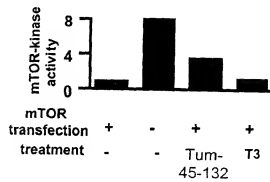


Fig. 61E

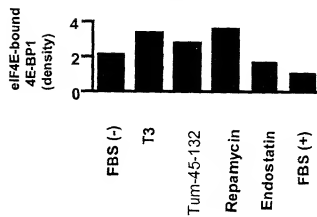
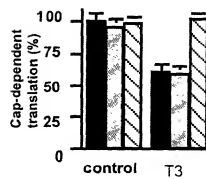


Fig. 61F



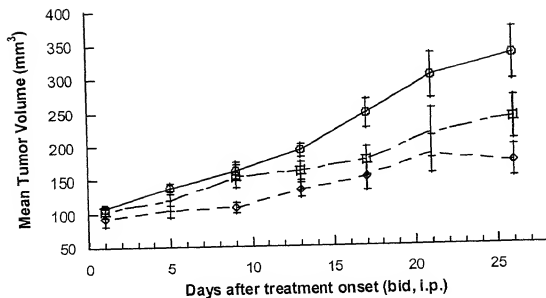


Fig. 62

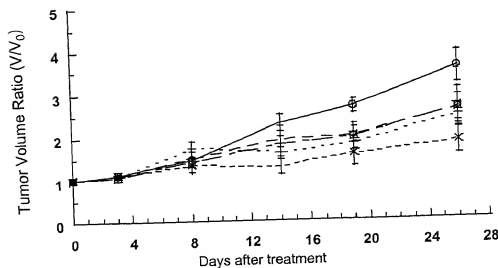


Fig. 63

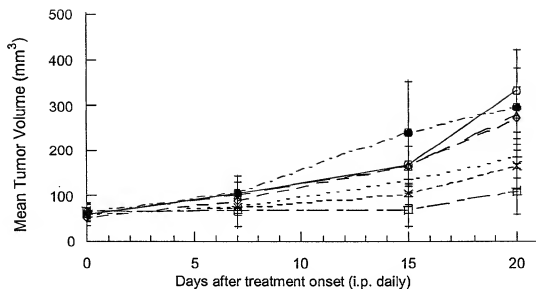


Fig. 64A

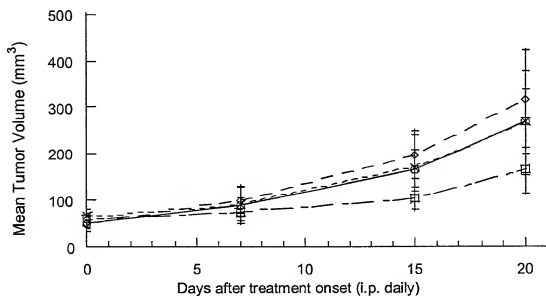


Fig. 64B

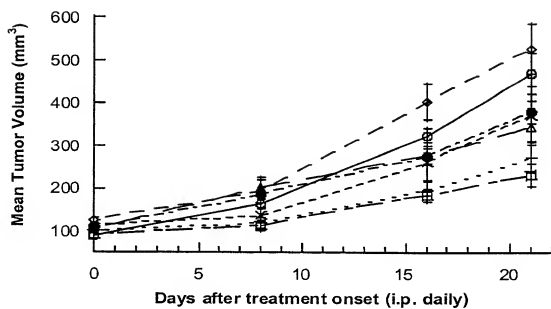


Fig. 65A

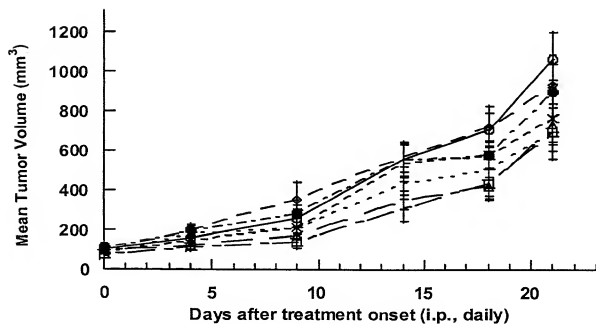


Fig. 65B